The volatile leaf oils of *Juniperus flaccida* Schltdl., *J. martinezii* Pérez de la Rosa and *J. poblana* (Mart.) R. P. Adams, re-examined

Robert P. Adams
Biology Department, Baylor University, Box 97388, Waco, TX 76798, USA Robert_Adams@baylor.edu

and

Thomas A. Zanoni
New York Botanical Garden, 200th Street at Southern Blvd.
Bronx, NY 10458-5126

ABSTRACT

The volatile leaf oils of *J. flaccida, J. martinezii and J. poblana* are re-examined and the composition is published using FID quantitation. The leaf oil of *J. flaccida* is dominated by α-pinene (65.0%) with moderate amounts of β-pinene (4.8%), myrcene (4.3%), limonene (3.5%), β-phellandrene (3.4%), linalool (2.9%) and manool oxide (3.5%). The oil of *J. poblana* is somewhat similar as it is dominated by α-pinene (52.9%) with moderate amounts of β-pinene (4.2%), myrcene (4.3%), limonene (2.2%), β-phellandrene (3.5%) and linalool (1.9%), but contains several unique compounds: δ-2-carene (1.8%), δ-3-carene (1.4%), trans-verbenol (2.7%), methyl chavicol (0.7%), and (E)-nerolidol (2.5%). The oil of *J. martinezii* was quite distinct with major components being α-pinene (16.6%), sabinene (10.4%) and camphor (11.1%) and moderate amounts of β-pinene (1.4%), myrcene (3.6%), limonene (1.8%), β-phellandrene (5.3%),linalool (2.8%), γ-terpinene (1.8%) and terpinen-4-ol (6.1%). It also contain several unique compounds: p-cymenene (0.7%), karahanaenone (1.3%), trans-dehydrocarvone (0.6%), trans-chrysanthenyl acetate (0.5%), linalool acetate (0.4%), noe0iso-3-thyjanyl acetate (0.8%), an aromatic phenol (KI 1320, 0.5%), trans-muurola-4(14), 5-diene (0.7%), epi-cubebol (0.5%), cubebol (1.1%), 1-epi-cubebol (1.0%), and an unknown diterpene (KI 1978, 0.6%). Published on-line **www.phytologia.org** *Phytologia 97(2): 145-151 (April 1, 2015)*. ISSN 030319430.

KEY WORDS: *Juniperus flaccida, J. martinezii, J. poblana, Cupressaceae*, terpenes, leaf essential oil.

The taxonomy of *Juniperus flaccida* Schltdl. has been somewhat unsettled. Generally, flaccid (weeping) foliaged junipers in Mexico (and the Chisos Mtns., Texas) have been referred to as *J. flaccida*. The first systematic treatment of these junipers was by Martinez (1963) who recognized two varieties: *J. f.* var. *typica* and *J. f.* var. *poblana* Mart. *Juniperus f.* var. *flaccida* has large seed cones (9-20 mm), with 6-10 seeds, and pendulous (flaccid) foliage and branchlets. *Juniperus f.* var. *poblana* also has large seed cones (9-12 mm), with 6-10 seeds, but the foliage is distichous and in vertical planes like *Thuja*, and not very flaccid (Zanoni and Adams, 1976, 1979; Adams, 2014). Pérez de la Rosa (1985) discovered a population of trees that had small seed cones (5-7 mm), with 1-2 seeds per cone and with foliage somewhat drooping but branchlets erect. He described this taxon as a new species, *J. martinezii* Pérez de la Rosa. Except for the seed cones, the taxon looks similar to *J. flaccida*; indeed Silba (1985) treated it as *J. flaccida* var. *martinezii* (Pérez de la Rosa) Silba. Each of these varieties has leaf margins that are hyaline and nearly entire, with either a few small teeth or merely a wavy margin (Adams, 2014). However, they are considered part of the serrate leaf margined *Juniperus* species of the western hemisphere (Adams, 2014).

Adams et al. (1990) compared the leaf essential oils and found considerable differences among the *J. flaccida* varieties. However, they decided to accept *J. flaccida* var. *martinezii* until "...additional data, such as from DNA analysis, are available." (Adams et al. 1990).

DNA sequencing of nrDNA (ITS) and trnC-trnD (Adams, et al. 2006) revealed that *J. flaccida* varieties are not monophyletic and they recognized *J. martinezii* and *J. f.* var. *poblana* as *J. poblana* (Mart.) R. P. Adams. More recently, Adams and Schwarzbach (2013) published a detailed phylogeny of the serrate junipers of the western hemisphere based on nrDNA and four cp genes. They found *J. flaccida* (var. *flaccida*) in a clade with *J. standleyi* (Fig. 1) and *J. poblana* (*J. f.* var. *poblana*) in a well supported sister clade. *Juniperus martinezii* (*J. f.* var. *martinezii*) was found in a well supported clade with *J. durangensis* (Fig. 1). Their work appears to solidify support for the recognition of *J. martinezii* and *J. poblana.*

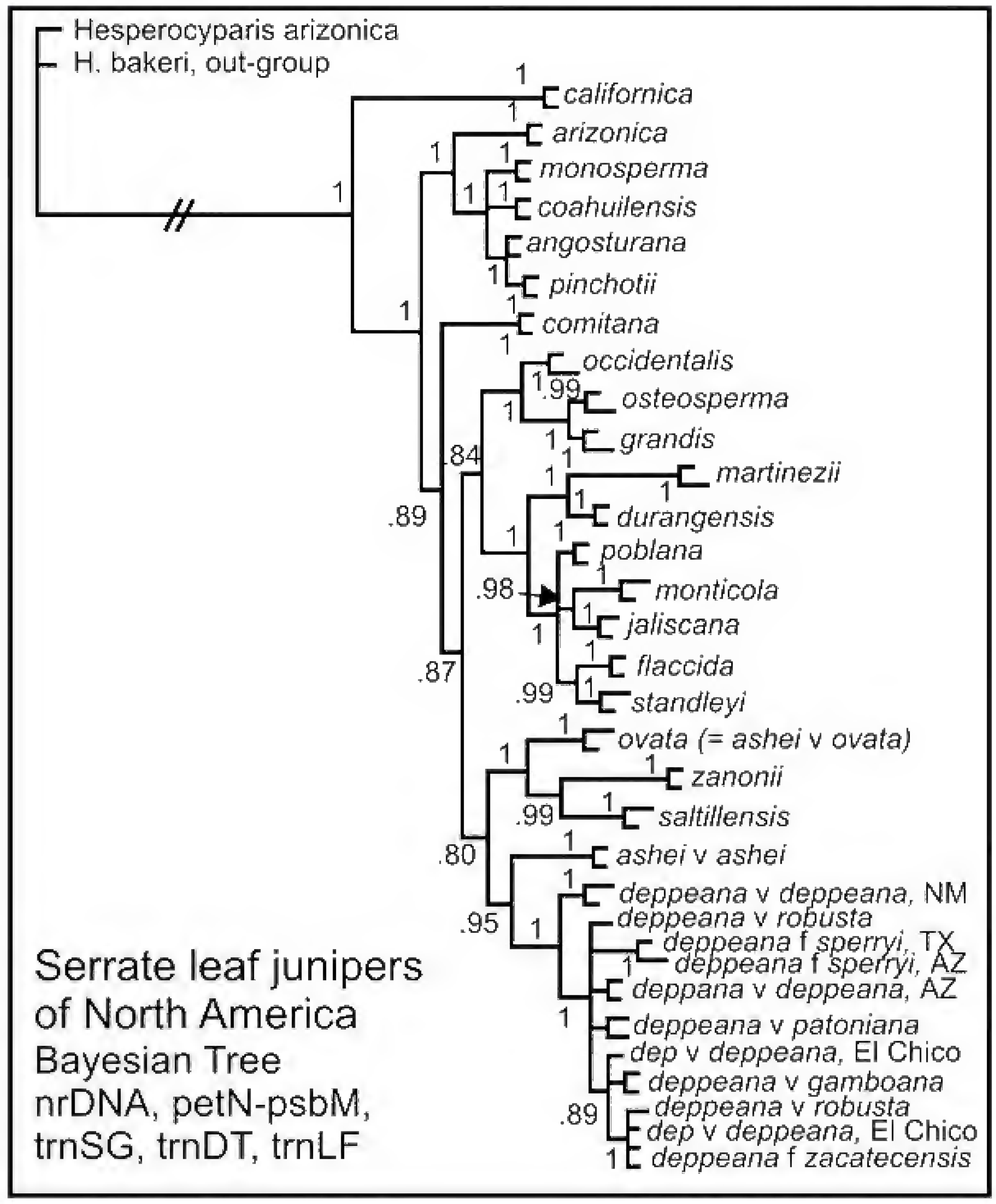

Figure 1. Bayesian analysis of the serrate leaf junipers of North America. Numbers are posterior probabilities. From Adams and Schwarzbach (2013). See text for discussion

The composition of the volatile leaf oils of *J. flaccida* and *J. f.* var. *poblana* were first reported by Adams, Zanoni and Hogge (1984). The composition of the leaf oil of *J. martinezii* was reported by Adams, Pérez de la Rosa and Charzaro (1990). However, these reports presented the data on a TIC (total ion count) basis, but, currently, the use of FID (flame ionization detector) basis is preferred as being more accurate.

The purpose of this paper is to report on a new analysis of the leaf oils of *J. flaccida, J. martinezii and J. poblana* using FID quantitation plus a library of over 2,205 compounds (Adams 2007) for identification.

MATERIALS AND METHODS

Specimens collected: *J. flaccida* var. *flaccida*, *Adams 6892-6896*, 23 km e of San Roberto Junction on Mex. 60, Nuevo Leon, Mexico; *J. martinezii*, *Adams 5950-5952, 8709*, 40 km n of Lago de Moreno on Mex. 85 to Amarillo, thence 10 km e to La Quebrada Ranch, 21° 33.08' N, 101° 32.57' W, Jalisco, Mexico; *J. flaccida* var. *poblana*, *Zanoni 2637-2643*, 0.74 mi N of Amozo on old Rt. 150, Puebla, MX; *Adams 6868-6870*, 62 km s of Oaxaca, Mexico on Mex. 190. Voucher specimens are deposited at BAYLU.

Fresh, air dried leaves (50-100 g) were steam distilled for 2 h using a circulatory Clevenger-type apparatus (Adams, 1991). The oil samples were concentrated (ether trap removed) with nitrogen and the samples stored at 20 ºC until analyzed. The extracted leaves were oven dried (100 ºC, 48 h) for determination of oil yields.

Oils from 4-5 trees of each taxon were analyzed and average values reported. The oils were analyzed on a HP 5971 MSD mass spectrometer, scan time 1/ sec., directly coupled to a HP 5890 gas chromatograph, using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column (see Adams, 2007 for operating details). Identifications were made by library searches of our volatile oil library (Adams, 2007), using the HP Chemstation library search routines, coupled with retention time data of authentic reference compounds. Quantitation was by FID on an HP 5890 gas chromatograph using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column using the HP Chemstation software.

RESULTS AND DISCUSSION

Overall, the leaf oils of *J. flaccida* and *J. poblana* are similar and the oil of *J. martinezii* is quite different. The leaf oil of *J. flaccida* is dominated by α-pinene (65.0%) with moderate amounts of β-pinene (4.8%), myrcene (4.3%), limonene (3.5%), β-phellandrene (3.4%), linalool (2.9%) and manool oxide (3.5%). The oil of *J. poblana* is somewhat similar as it is dominated by α-pinene (52.9%) with moderate amounts of β-pinene (4.2%), myrcene (4.3%), limonene (2.2%), β-phellandrene (3.5%) and linalool (1.9%), but contains several unique compounds: δ-2-carene (1.8%), δ-3-carene (1.4%), trans-verbenol (2.7%), methyl chavicol (0.7%), and (E)-nerolidol (2.5%). The oil of *J. martinezii* was quite distinct with major components being α-pinene (16.6%), sabinene (10.4%) and camphor (11.1%) and moderate amounts of β-pinene (1.4%), myrcene (3.6%), limonene (1.8%), β-phellandrene (5.3%),linalool (2.8%), γ-terpinene (1.8%) and terpinen-4-ol (6.1%). It also contain several unique compounds: p-cymenene (0.7%), karahanaenone (1.3%), trans-dehydrocarvone (0.6%), trans-chrysanthenyl acetate (0.5%), linalool acetate (0.4%), noe-iso-3-thyjanyl acetate (0.8%), an aromatic phenol (KI 1320, 0.5%), trans-muurola-4(14), 5-diene (0.7%), epi-cubebol (0.5%), cubebol (1.1%), 1-epi-cubebol (1.0%), and an unknown diterpene (KI 1978, 0.6%).

ACKNOWLEDGEMENTS

Thanks to Amy Tebeest for lab assistance. This research was supported in part with funds from Baylor University.

LITERATURE CITED

Adams, R. P. 1991. Cedarwood oil - Analysis and properties. pp. 159-173. in: Modern Methods of Plant Analysis, New Series: Oil and Waxes. H.-F. Linskens and J. F. Jackson, eds. Springler- Verlag, Berlin.

Adams, R. P. 2007. Identification of essential oil components by gas chromatography/ mass spectrometry. 4th ed. Allured Publ., Carol Stream, IL.

Adams, R. P. 2014. The junipers of the world: The genus *Juniperus*. 4th ed. Trafford Publ., Bloomington, IN.

Adams, R. P., J. A. Pérez de la Rosa and Miguel Charzaro B. 1990. The leaf oil of *Juniperus martinezii* Pérez de la Rosa and taxonomic status. J. Essential Oils Res. 2: 99-104.

Adams, R. P. and A. E. Schwarzbach. 2013. Taxonomy of the serrate leaf *Juniperus* of North America: Phylogenetic analyses using nrDNA and four cpDNA regions. Phytologia 95: 172-178.

Adams, R. P., A. E. Schwarzbach and S. Nguyen. 2006 Re-examination of the taxonomy of *Juniperus flaccida* var. *martinezii*, and var. *poblana* (Cupressaceae) Phytologia 88: 233-241.

Adams, R. P., T. A. Zanoni and L. Hogge. 1984. The volatile leaf oils of *Juniperus flaccida* var. *flaccida* and var. *poblana*. J. Natl. Prod. 47:1064-1065.

Martinez, M. 1963. Las pinaceas mexicanas. Tercera edicion. Universidad Nacional Autonoma de Mexico. Mexico City.

Pérez de al Rosa, J. A. 1985. Una nueva especie de *Juniperus* de Mexico. Phytologia 57: 81-86.

Silba, J. 1985. A supplement to the international census of the coniferae. I. Phytologia 58: 365-370.

Zanoni, T. A. and R. P. Adams. 1976. The genus *Juniperus* (Cupressaceae) in Mexico and Guatemala: Numerical and chemosystematic analysis. Biochem. Syst. Ecol. 4: 147-158.

Zanoni, T. A., Adams, R. P. 1979. The genus *Juniperus* (Cupressaceae) in Mexico and Guatemala: Synonymy, Key, and distributions of the taxa. Bol. Soc. Bot. Mexico 39: 83-121.

Table 1. Leaf essential oil compositions for *J. flaccida* (*Adams 6892*), *J. martinezii* (*Adams 5974*), and *J. poblana* (*Adams 2578*), based on FID gas chromatography. KI = Kovats Index (linear) on DB-5 column. Compositional values less than 0.1% are denoted as traces (t). Unidentified components less than 0.5% are not reported. Those compounds that appear to distinguish taxa are in boldface.
* = Tentatively identified.

KI	Compound	*flaccida*	*poblana*	*martinezii*
921	**tricyclene**	**0.2**	**t**	**0.6**
924	**α-thujene**	**t**	**t**	**0.6**
932	**α-pinene**	**65.0**	**52.9**	**16.6**
945	α-fenchene	t	0.1	-
946	camphene	0.6	0.7	0.7
953	thuja-2,4-diene	t	0.2	0.1
961	verbenene	1.3	0.6	0.2
969	**sabinene**	**0.2**	**0.2**	**10.4**
974	1-octen-3-ol	-	-	-
974	**β-pinene**	**4.8**	**4.2**	**1.4**
988	myrcene	4.3	4.3	3.6
1001	**δ-2-carene**	**-**	**1.8**	**-**
1001	4-methyl, me-pentanoate*	0.1	-	-
1002	α-phellandrene	0.1	0.1	1.0
1008	**δ-3-carene**	**-**	**1.4**	**-**
1014	α-terpinene	t	t	1.0
1020	p-cymene	0.1	0.2	1.8
1024	limonene	3.5	2.2	1.8
1025	β-phellandrene	3.4	3.5	5.3
1032	(Z)-β-ocimene	t	t	t
1044	(E)-β-ocimene	1.5	0.7	0.4
1054	**γ-terpinene**	**0.2**	**0.1**	**1.8**
1065	cis-sabinene hydrate	-	-	0.6
1067	cis-linalool oxide (furanoid)	0.1	t	-
1086	terpinolene	0.5	0.7	0.8
1089	**p-cymenene**	**-**	**-**	**0.7**
1092	96, 109,43,152, C10-OH	1.0	0.3	1.8
1095	**linalool**	**2.9**	**1.6**	**2.8**
1112	3-m-3-buten-me-butanoate	0.2	-	-
1114	endo-fenchol	-	0.3	-
1118	cis-p-menth-2-en-1-ol	0.1	0.2	0.5
1122	α-campholenal	0.3	1.2	0.4
1133	cis-p-mentha-2,8-dien-1-ol	-	t	-
1135	trans-pinocarveol	0.3	1.1	0.8
1136	trans-p-menth-2-en-1-ol	-	-	-
1141	**camphor**	**0.5**	**0.6**	**11.1**
1141	**trans-verbenol**	**-**	**2.7**	**-**
1145	camphene hydrate	0.4	0.5	1.3
1148	citronellal	0.2	t	-
1154	**karahanaenone**	**-**	**-**	**1.3**
1155	iso-isopulegol	0.1	-	-
1160	**p-mentha-1,5-dien-8-ol**	**-**	**0.5**	**1.0**
1165	**borneol**	**0.7**	**0.6**	**-**
1172	cis-pinocamphone	0.2	0.3	0.3
1174	**terpinen-4-ol**	**0.3**	**0.3**	**6.1**
1178	**naphthalene**	**-**	**t**	**t**
1179	**p-cymen-8-ol**	**t**	**t**	**0.5**
1186	α-terpineol	0.4	0.7	0.7
1195	myrtenol	0.1	0.2	t
1195	myrtenal	-	-	0.1

KI	Compound	*flaccida*	*poblana*	*martinezii*
1195	**methyl chavicol**	**-**	**0.7**	**-**
1200	trans-dehydrocarvone	-	-	0.6
1204	**verbenone**	**t**	**0.6**	**0.5**
1215	**trans-carveol**	**0.1**	**0.7**	**-**
1218	endo-fenchyl acetate	-	-	-
1223	citronellol	0.1	-	-
1232	thymol, methyl ether	-	-	-
1235	**trans-chrysanthenyl acetate**	**-**	**-**	**0.5**
1239	carvone	-	0.2	-
1249	**piperitone**	**0.2**	**0.9**	**0.9**
1254	**linalool acetate**	**-**	**-**	**0.4**
1255	4Z-decenol	0.2	-	-
1284	bornyl acetate	0.4	1.1	1.8
1289	trans-sabinyl acetate	-	-	0.1
1289	**neo-iso-3-thyjanly acetate**	**-**	**-**	**0.8**
1289	thymol	-	0.2	-
1292	(2E,4Z)-decadienal	0.1	-	-
1315	(2E,4E)-decadienal	0.1	-	-
1320	**aromatic phenol 149,91,77,164**	**-**	**-**	**0.6**
1344	myrtenyl acetate	-	0.1	-
1345	α-terpinyl acetate	-	-	0.2
1345	α-cubebene	0.1	0.1	0.3
1396	duvalene acetate	-	-	-
1403	methyl eugenol	0.1	-	-
1417	**(E)-caryophyllene**	**0.2**	**0.3**	**0.1**
1448	cis-muurola-3,5-diene	-	-	-
1451	trans-muurola-3,5-diene	-	-	0.2
1452	α-humulene	-	t	-
1475	trans-cadina-1(6),4-diene	-	-	0.3
1484	**germacrene D**	**0.1**	**0.3**	**-**
1493	**trans-muurola-4(14),5-diene**	**-**	**-**	**0.7**
1493	**epi-cubebol**	**-**	**-**	**0.5**
1500	α-muurolene	-	t	-
1513	γ-cadinene	-	-	-
1514	**cubebol**	**-**	**-**	**1.1**
1521	trans-calamenene	-	t	0.5
1522	δ-cadinene	-	t	0.4
1528	zonarene	-	-	0.1
1533	trans-cadina-1,4-diene	-	-	t
1548	elemol	0.1	0.2	1.0
1555	elemicin	-	0.2	-
1561	**(E)-nerolidol**	**-**	**2.5**	**-**
1582	caryophyllene oxide	0.2	0.6	0.3
1627	**1-epi-cubenol**	**-**	**-**	**1.0**
1630	γ-eudesmol	-	-	t
1638	epi-α-cadinol	-	0.1	-
1638	epi-α-muurolol	-	0.1	-
1649	β-eudesmol	-	t	0.3
1652	α-eudesmol	-	0.1	0.3
1652	α-cadinol	-	0.1	-
1685	germacra-4(15),5,10-triene-1-al	-	-	-
1759	benzyl benzoate	-	-	-
1933	cyclohexadecanolide	-	-	-
1958	**iso-pimara-8(14),15-diene**	**0.1**	**-**	**1.0**
1978	**diterpene,43,81,147,243**	**-**	**-**	**0.6**
1987	manoyl oxide	3.0	0.3	1.0
2055	abietatriene	0.3	0.2	0.8

KI	Compound	*flaccida*	*poblana*	*martinezii*
2087	**abietadiene**	**-**	**-**	**2.3**
2056	manool	-	-	-
2105	iso-abienol	-	0.1	-
2264	**diterpene,43,55,271,286**	**-**	**t**	**-**
2331	trans-ferruginol	-	t	-